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DISCAR

AN EXPERIMENTAL STUDY
ON THE EFFECT OF THE
Blood Sera of Normal and Immunized Goats
IN MODIFYING THE PROGRESS OF
TUBERCULOUS INFECTION

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1903

I.—*An Experimental Study on the Effect of the Blood-sera of Normal and Immunized Goats in Modifying the Progress of Tuberculous Infection.*

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From the J. H. R. Molson Laboratories of Pathology.

(Communicated by Professor J. G. Adami, M.D., and read May 19, 1903.)

DISCARD

One of the most important subjects, if not indeed the most important, occupying the attention of the experimental investigator in the domain of medicine at the present time, is the discovery of some curative agent to be employed in that most dread and widespread disease to which human beings are subject, tuberculosis. For the past fifteen years some of the brightest intellects have been at work on this problem with as much zeal and hope, albeit let us confess with more knowledge, as was ever expended by the philosophers of old in the search for the "Philosopher's Stone" or the "Elixir of Life." We have, however, reluctantly to admit, that the collective result of these most searching and extensive investigations, while beyond doubt it has increased our knowledge of the nature of the tubercle bacillus and the morbid processes induced by it, has lead as yet to but little practical advance. The final solution of the problem has hitherto eluded our grasp, and investigator after investigator has, like his prototype the more dramatic alchymist of the dark ages, seen the golden vision fade from his eyes when the prize was thought to be won.

The brilliant success that has crowned the efforts of experimenters to produce an antitoxic serum for diphtheria and hydrophobia, and the somewhat less valuable results that have been attained in tetanus, septicæmia, and typhoid fever, have excited the justifiable hope that a similar remedy might be devised in tuberculosis. Here, however, the problem has turned out to be by no means as simple as in the case of the diseases just mentioned. Practical experience has shown that the efficacy of the antitoxic sera hitherto prepared is in direct ratio to the virulence of the diseases in which they are employed. Unlike diphtheria tuberculosis is not a self-limited disease, nor does it kill by septicæmia. It belongs to that group of diseases to which leprosy, syphilis,

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The experiments detailed in the following paper were begun on the initiative of the Hon. E. H. Bronson, of Ottawa, who has generously borne the expense of the investigation, and has evinced throughout the most kindly interest in the work.

and actinomycosis also belong, that have a notably slow progression, presenting, it is true, effects referable to a mild intoxication, but also characterized by the production of gross organic lesions (granulomata) in various parts. Here it is obvious that a purely antitoxic serum, that is to say, one that merely neutralizes the poisons elaborated by the tubercle bacillus in the course of its growth, is hardly likely to prove effective. Should it be possible to prepare such a serum, and, as we shall see, it undoubtedly is, the disease would still progress. To be of real value a serum would apparently need to possess germicidal as well as antitoxic properties. Even in the days of the discovery of the diphtheria antitoxin and before (1884), attempts were made to solve the problem and the names of Héricourt and Richet stand out as pioneers in this line of research. Since then the number of workers has increased marvellously, and the work of Koch, Maragliano, Babès, Maffuci and Di Vestea, Behring, Niemann, and on this continent, Trudeau, and DeSchweinitz, is a monument of painstaking research, scientific accuracy, and devotion to truth. No one who has not investigated the subject would credit the enormous amount of labour expended on this one problem. A glance at the bibliography appended to this paper, which represents merely one phase of the subject, namely that relating to animal sera, will emphasize the truth of this remark.

The subject has been attacked in various ways. One class of investigator has attempted the cure of tuberculosis by means of drugs, a mode which seems again of late to be coming into prominence. A second school, of which Koch has for some years been the leading spirit, has sought to produce immunity and cure the disease by the injection of various toxins derived from the tubercle bacillus or chemical modifications thereof. To this category of remedies belong the various tuberculins, oxytuberculin, tuberculocidin, and antiphthisin. By the injection of these substances it is sought to stimulate the cells of the body to the elaboration of an antitoxic substance in such amount as to neutralize the poisons eliminated by the bacilli. The third series of experimenters has endeavored by the injection of various extracts of tubercle bacilli or in some cases the living attenuated germs, to produce immunity in certain of the lower animals, and then to use the blood serum of animals thus fortified to combat the disease in other individuals. It is with work of this last class that this paper alone will deal.

The first observation, and one that has led to all the rest, was that of Héricourt and Richet. In 1888 they noted that if a rabbit, which is very susceptible to this germ, be inoculated with the staphylococcus pyosepticus, it may be rendered refractory to its action by intraperitoneal injection of dog's blood, an animal that possesses a natural immunity to the infection. This suggested to their minds that the same thing might hold good in tuberculosis. Without entering into details, the chief conclusions to which they arrived were (1) that in animals the subject of experimental tuberculosis the injection of dog's blood will arrest the disease provided the germ be not too virulent, or will retard it if it be very virulent. (2). The serum of a dog injected into a healthy rabbit will prevent the development of experimental tuberculosis subsequently. (3) The serum of a dog previously inoculated with tuberculosis when injected into rabbits already tuberculized will aggravate the disease. They did not believe that dog's serum possessed a specific curative action in tuberculosis, although it seemed to have a powerful action against some of the effects of the germ and exerted a tonic action on nutrition (3.) Extract of dog's liver possessed similar properties. In some of their later work they employed the ass (36). The blood of the normal ass, like that of the dog, was found to retard the tuberculous process, but the serum of an ass previously injected with tubercle bacilli proved to be still more potent.

The special merit of Héricourt and Richet lies in the fact that they were the first to see the possibility of producing by the injection of tubercular virus a specific means of combatting the disease. It was Maragliano (17), however, who first demonstrated by scientific proofs that a tubercular antitoxin did exist and applied it to the treatment of human tuberculosis. His results were given to the French Congress of Medicine at Bordeaux in 1895, and his subsequent investigations have proved to be so brilliant and painstaking that a brief reference here will not be out of place.

Koch's original tuberculin, at one time used largely for therapeutic purposes, was prepared by heat, so that it did not represent the full toxic properties of the bacillus, for, as Auclair has proved, certain volatile poisons are given off in the course of preparation. These substances have been shown by Maragliano to cause convulsions when injected into rabbits. The glycerine also used in the preparation is toxic. Maragliano therefore employs a watery extract of bacilli. He makes use of two toxins: A., prepared by concentrating a culture of the bacilli over a water-bath at 100°C. for three or four days; and B., a culture filtered through a Chamberland filter at the room temperature and concentrated in a vacuum at a temperature less than 30°C. For inoculation purposes he uses three parts of A. and one of B. This mixture is injected into horses beginning with a dose of two milligrammes per kilo. of the body weight, increased gradually one milligramme a day up to forty or fifty. The injections are stopped if the animals show signs of fever or emaciation until recovery has taken place. The whole process is spread over about six months. Before using the horse serum thus prepared Maragliano waits three or four weeks until the urine is free from toxic bodies. One c. cm. will counteract the smallest dose of tuberculin that will produce a reaction in a tuberculous man. The serum is said also to possess appreciable germicidal properties. Maragliano has knowledge of 1,362 tuberculous patients treated by his serum. Eighty per cent were benefited by it. The most favorable results were obtained in afebrile cases with localized lesions. In cases of mixed infection the serum was less useful. Maragliano has also shown that the serum of tuberculous patients treated with his serum was two to three times as antitoxic as it was before the injections.

Maffucci and Di Vestea (56), have attempted to attain the same end by using sheep, which are supposed to be refractory to tuberculosis, employing the methods of Héricourt and Richet and the "mithridatization" method with Behring's antitoxin. They injected both dead and living bacilli into the sheep, but found that the serum resulting was neither curative nor prophylactic, but at most caused some retardation of the disease. They found moreover that while the serum was innocuous for guinea-pigs in doses of two c. cm. per hundred grammes, one-fifth of a c. cm. in rabbits produced a fatal hæmoglobinuria. When added to a culture of tubercle bacilli in the proportion of four to one some attenuation of the germs was produced.

Niemann (69) has used goats. He injected for some weeks a tuberculin derived from a very virulent stock of bacilli until he was giving fifteen c. cm. Then he injected an alcoholic precipitate from tuberculin that had been proved to be extremely toxic, beginning at first with twelve to eighteen milligrammes and increasing after a month to half to one gramme. He found that by the use of the antitoxic serum thus prepared he could prolong the disease and claimed to have observed good results in human beings.

The results of DeSchweinitz and Dorset (80), are somewhat similar. They inoculated cows and horses with tuberculin and bacilli and found that this conferred on their sera some powers of retarding the disease in guinea-pigs. The serum of cows inoculated with attenuated bacilli proved to be more potent.

Trudeau and Baldwin (105), were able to produce a marked degree of immunity in rabbits by the injection of attenuated cultures but the serum of such animals did not appear to have gained antitoxic properties. In a large number of experiments with sheep, asses, rabbits, and chickens inoculated with living germs, they thought that the sera thus fortified possessed slight antitoxic properties. Their results were, however, not very convincing.

From the above mentioned observations it will be gathered that the results of experiment are by no means uniform and not entirely satisfactory. The best results have been obtained where in addition to the toxins the substance of the germs has been employed as well, but even in this case there is some discrepancy between the different observers. In fact Maragliano is the only one who seems to be at all sanguine as to the value of his serum.

The few observations I have made have been carried out on slightly different lines from those hitherto published in the hope that they would throw some light on a somewhat doubtful question and possibly elicit some new facts. I have employed goats for the purpose, finding them on the whole the most suitable. It used to be thought that goats were absolutely refractory to tuberculosis but this is certainly not the case. (See Colin. Compt. rend. Acad. de Sc., Paris, 1891, CXIII, 219). It is true, however, that in a natural state they rarely suffer from the disease. It is, however, possible to give them the disease by injecting living virulent bacilli in considerable quantities into the circulation, although subcutaneous injection will not suffice. They are therefore relatively immune. Being also hardy animals they stand the various manipulations well. A considerable quantity of serum can be obtained from them which is of good quality and keeps well. Goat serum has the further advantage, as Lépine has shown (Sém. méd. 1891, XI., 21), that it produces much less hæmolysis when added to human blood than does dog's serum. In spite of many advantages goats have not been used to any great extent in this kind of work. Bertin and Picq, Boinet and Niemann are the most noteworthy of those who have done so and report good results. Bertin and Picq (6) found that rabbits previously injected with goat's blood were able to resist a subsequent infection with tuberculosis, although they state that the injections had no effect in animals already tuberculized.

THE EFFECTS OF NORMAL GOAT SERUM.

It is now a well recognized fact that the sera of several normal animals possess what may be termed natural antitoxic bodies, or perhaps more correctly alexins. Thus, horse serum possesses about two or three hundred antitoxic units against the diphtheria germ, and according to Maragliano human serum possesses three to four hundred units against the bacillus of tuberculosis. It was thought advisable, therefore, to determine first whether normal goat serum could in any sense be considered protective against tuberculosis. Should this prove to be the case, one might then try to increase this up to a practical point.

For the purposes of the experiment it was obviously necessary to obtain the serum without contamination from bacteria and as nearly normal as possible. To attain this the following method was adopted. A large healthy male goat was taken, the hair was removed over the course of the external jugular vein in the neck, and the skin washed and sterilized by means of a solution of sublimate (1—1000). A large sterilized trocar attached by a rubber tube to a sterilized bottle was then inserted into the vein and the blood allowed to flow into the vessel. The serum was allowed to separate in a cold chamber, the clear portion carefully decanted off, and one-quarter per cent of chloroform added as a preservative. It was found that the serum thus prepared kept perfectly well for some weeks.

Experiment I.

The first experiment was conducted under the following conditions: Eight guinea-pigs and ten rabbits, presumably in good health were taken, and their weight and temperature before inoculating were ascertained. They were then numbered and kept in separate hutches. On March 13th, 1902, they were inoculated, one-half intraperitoneally and the other under the skin of the left leg, with a culture of the bacillus tuberculosis of extremely mild virulence, standardized as follows:—

A culture of the Bacillus Typhi Abdominalis, taken from old laboratory stock, was inseminated in 1.5% acid broth and grown in the incubator at the usual temperature for twenty-four hours. The culture obtained was then killed with formalin vapour and used as a standard. A glycerine-agar culture of the tubercle bacillus referred to was ground up in a sterile mortar with sterile normal saline solution. This was allowed to stand until the heavier portions had sunk to the bottom. The opalescent supernatant portion was carefully decanted off and diluted with sterile normal salt solution until it reached the same degree of opacity as the standard culture of the B. Typhi. Hanging-drops were then examined under the microscope to see that there were no gross masses of bacilli floating about. One cubic centimetre of this material was then used for inoculating. Care was, of course, taken as far as possible to avoid contamination in the course of the various manipulations, sterilized vessels and instruments being invariably employed.

The animals to be injected were shaved at the desired points and the skin sterilized with bichloride, 1—1,000. The inoculations were made with an all-metal syringe of five c. cm. capacity, previously boiled.

The reason for using a culture of weak virulence to begin with was that guinea-pigs are very susceptible to tuberculosis and it was inferred from observations already referred to, that should goat serum possess any antitoxic powers these would be extremely slight.

One-half of the animals were inoculated subcutaneously over the abdomen with two c. cm. of normal goat-serum every second day. Subsequently the temperatures were taken every day and the weights once a week. Instead of estimating, as others have done, the effect of the injections by keeping the animals until they died spontaneously, and taking into consideration merely the loss of weight, it was thought advisable, as we were dealing with germs of such mild virulence and there was a possibility of the animals recovering, to kill them at stated intervals and determine the amount of tuberculosis by the naked eye and the microscope. By this method an exact appreciation of the state of things could be obtained. By arranging them in pairs according to weight it was also possible to compare animals of approximately the same degree of resisting power.

The subjoined table will give at a glance the more important features of the experiment:—

NOS.	T. BEFORE INOC.	T. AFTER.	WT. BEFORE INOC.	WT. AFTER.	REMARKS.
G—Pig.					
I.	100·8	101·8	425 grms.	477·5	Minimal tbc.
IV.	*99·2 ?*	*103*	*420*	*443*	? lymphatic tbc.
II.	98 ?	101·4	454	452·7	Tbc. of testis
VI.	*102·4*	*103*	*443*	*480·5*	Local tbc. in abdom. wall.
III.	101·6	102·9	489	525	Slight tbc.
VII.	*101·8*	*102·4*	*349*	*377·2*	Slight tbc.
V.	102	102·4	327	399	? tbc.
VIII.	*100*	*102·6*	*217*	*232*	No positive tbc.
Rab.					
I.	*102·5*	*101·4*	*1880*	*1431*	No tbc.
VI.	100·4	102·4	1432	1396	No tbc.
V.	*101·4*		*1556*		No tbc.
IX.	*100·2*	*103·2*	*2142*	*1790*	No tbc.
VIII.	100·8		1475		Tbc. of lung and pleura.
II.	102·4	101·6	1847	1667	No tbc.
VII.	*100·6*	*103·3*	*1102*	*1322*	No tbc.
IV.	101·6	101·9	1932	1569	No tbc.
X.	*109·6*		*1510*		Minimal local tbc.
III.	102·8	102·1	2101	1646	No tbc.

N. B.—In the above table the numbers in italics were given normal goat serum The weights and temperatures after inoculation are average. The animals are grouped in pairs according to weight.

In the above table I do not lay much stress upon the weights and temperatures before inoculation inasmuch as observations were not made for a sufficiently long time before the experiment was begun.

Two guinea-pigs and two rabbits died spontaneously before the conclusion of the experiment, apparently from some gastro-intestinal disturbance. The rest of the pigs were killed after thirty days, and one-half of the remaining rabbits about the same time. The first animals killed presented so little pathological change that it was thought advisable to keep the remaining six for two weeks longer in the hope that the lesions would be more marked. Autopsies were performed at various times with the special object of determining the extent of the dissemination of the tuberculous virus, the effect of the serum injections, if any, and the character of the bacilli of tuberculosis found in the various parts. Portions of the organs were examined microscopically, both by the hæmatoxylin method and the modified Ziehl-Nielsen method for tubercle bacilli. Smears were made from the organs and stained for bacteria. Cultures were also taken from the organs.

Abstracts of the autopsy protocols are herewith presented:—

GUINEA-PIG I.

Inoculated March 13th with 1 c.cm. of standard dilution of B. Tuberculosis intraperitoneally. Killed April 15th. Abdominal wall at site of inoculation red and inflamed, containing a small caseous mass. Three inguinal glands on the left side enlarged, a smear from which gave tubercle bacilli. *Spleen*: large, soft and pale. Other organs normal. Microscopical examination showed the organs to be congested, but without obvious tuberculosis. The *inguinal glands* showed inflammatory hyperplasia. Cultures from the heart-blood, bile and liver were sterile after thirty days.

GUINEA-PIG II.

Inoculated intraperitoneally. Died on twenty-fifth day. One or two small nodules, not caseous, in the abdominal wall at the site of inoculation. One minute elevated spot of yellowish colour on right testis and four or five similar ones on the left, surrounded by an inflammatory areola. Microscopically, these were composed of granulation tissue, small round-cells, and fibroblasts. Tubercle bacilli were demonstrated. Other organs normal. Cultures from heart-blood and spleen, sterile after thirty-four days.

GUINEA-PIG III.

Killed April 15th. Inoculated in left leg. Slight hæmorrhage in region of the inoculation wound. Two or three glands in the left inguinal region enlarged, soft, and caseous looking. Smears from these gave the B. Tuberculosis. Other organs normal. Cultures from liver, bile, and heart-blood sterile. Cultures from the affected glands and from the liver did not develop tubercle bacilli.

GUINEA-PIG IV.

Killed April 15th. Inoculated in peritoneal cavity. *Given goat serum*, 2 c.cm. every second day. Anterior abdominal wall intensely hæmorrhagic from the inoculations. Two or three inguinal glands on the left side, and a few mesenteric glands enlarge but films did not show tubercle bacilli. All organs normal. Cultures from the heart-blood, liver, and bile, sterile.

GUINEA-PIG V.

Killed April 15th. Inoculated in left leg. Slight hæmorrhage in region of original inoculation. The glands in the left inguinal region were enlarged and reddened, and a few mesenteric glands were swollen. Smears from mesenteric glands showed no tubercle bacilli. All organs normal. Cultures from inguinal glands, heart-blood, and bile, sterile.

GUINEA-PIG VI.

Killed April 15th. Inoculated intraperitoneally. Moderate hæmorrhage at site of inoculation of serum with a hæmatoma in the left inguinal region. At site of original inoculation the abdominal wall was thickened and contained a small caseous focus. All organs normal. Cultures heart-blood and bile, sterile. *Given serum.*

GUINEA-PIG VII.

Inoculated in left leg. *Given serum.* Great extravasation of blood into tissues of the thorax and abdominal wall. Glands of left inguinal region and one or two retroperitoneal glands on the left side enlarged, reddened, and the latter with some spots of opaque white. *Stomach* and *intestines* reddened and distended with gas. Other organs normal. Cultures from heart-blood and retroperitoneal gland, sterile; from liver, staphylcoccus albus.

GUINEA-PIG VIII.

Died on eighteenth day. Inoculated in left leg. *Given serum.* Site of serum injections in the abdominal wall infiltrated, reddened, and acutely inflamed. There was one small superficial focus with necrosis. The tissues over the thorax were much infiltrated. *Spleen*: swollen. *Kidneys*: congested with petechial spots. *Mesenteric glands*: enlarged and red, but not caseous. Cultures from mesenteric glands, sterile; from suprarenal, staphylococcus albus.

RABBIT I.

Inoculated in leg. *Given serum.* No local signs at point of inoculation, All organs absolutely normal.

RABBIT II.

Killed in about thirty days. Inoculated intraperitoneally. One mesenteric gland enlarged, not proved to be tuberculous.

RABBIT III.

Killed in about thirty days. No local signs at site of subcutaneous inoculation in abdomen. All organs normal except the liver which contained coccidia. A few mesenteric glands enlarged along the vessels as if from hæmatogenic infection, but films from these showed no tubercle bacilli.

RABBIT IV.

Inoculated intraperitoneally. Killed May 12th. No local signs at site of inoculation. All organs healthy looking. One or two enlarged glands noticed in the mesentery. Smears from these did not show tubercle bacilli.

RABBIT V.

Died in thirteen days. Inoculated in leg. *Given serum.* Abdominal and thoracic walls thickened, reddened, and very œdematous. One or two mesenteric glands slightly enlarged. *Kidneys*: cloudy. No signs of tuberculosis. Cultures from heart-blood, sterile.

RABBIT VI.

Inoculated in leg. No signs of tuberculosis.

RABBIT VII.

Inoculated intraperitoneally. *Given serum.* Slight congestion of the abdominal wall where the serum was given. All organs healthy.

RABBIT VIII.

Died in thirteen days. Inoculated in leg. No local signs in glands of groin. Upper lobe of left lung much shrunken, full of large scattered caseous masses. Caseous pleurisy with thickening. One or two minute tubercles in apex of right lung. Other organs normal. Peribronchial glands caseous.

RABBIT IX.

Inoculated in leg. *Given serum.* All organs healthy. No local signs of the inoculation of the serum.

RABBIT X.

Died on twenty-first day. Inoculated intraperitoneally. *Given serum.* Subcutaneous tissues over site of inoculation slightly œdematous and reddened. A small caseous yellow spot on inner side of abdominal wall at site of original inoculation. Condition of gastroenteritis present which had apparently caused death. No other signs of tuberculosis elsewhere. Cultures from the heart-blood and liver, sterile.

It will be seen from the foregoing that of the guinea-pigs only one (No. 2), gave evidence of any dissemination of the tubercle bacilli to a distance from the site of the original inoculation. It had not received serum. In Nos. 1 and 4 the inguinal glands were affected; the bacilli were recovered in No. 1 which had not received serum, not found in No. 4 which had. In No. 6, which had received serum, the infection was strictly localized to the site of inoculation. In those inoculated in the leg, viz., Nos. 3, 5, 7 and 8, all except one showed enlargement of the inguinal glands. In only one that had not received serum were the bacilli recovered (No. 3). One that had received serum did not develop a local lesion (No. 8).

In the case of the rabbits, only one developed gross tuberculosis (No. 8), and this one had not received serum. This result was in general what one would have expected as rabbits are much more refractory to tuberculosis than are guinea-pigs. None of the culture tubes developed the specific bacillus, and when found in smears, they were in a state of extreme fragmentation and degeneration, showing that the infection was an extremely mild one. So far as I could see the inoculations of serum had no effect whatever upon the temperature of the animals receiving it, but the rabbits so treated lost weight rather rapidly, although the pigs were unaffected. This was probably due to interference with the feeding, for the injections produced extensive areas of coagulation-necrosis in the abdominal wall and in one or two instances there was slight superficial suppuration. Apparently the injections of serum had some slight deterrent effect on the development of the tuberculous lesions, but it was felt that it was unwise to draw any positive conclusions from such a small series of animals, particularly with so mild a germ, so a second experiment was undertaken on similar lines but with several modifications suggested by the experience with the former series.

Experiment II.

Six guinea-pigs and twelve rabbits were placed under exactly the same conditions as to food, exercise, etc., and weighed at intervals of a week until an average normal weight was established. They were then grouped in pairs as nearly as possible according to the weight Rectal temperatures were taken daily for ten days to establish a mean normal temperature. Both the weights and the temperatures were found to vary in health between rather wide limits. The average temperature of the pigs was from 102° and 3-10ths to 102° and 8-10ths; that of the rabbits from 102° to 103° and 2-10ths.

All with the exception of two rabbits, which were retained as controls, were inoculated with one c. cm. of an emulsion of a more virulent, though still mild, culture of the tubercle bacillus in normal saline, standardized as before. One-half of the animals were given the inoculation in the left leg subcutaneously; the other half, intraperitoneally. Three days after inoculation one member of each pair was given a subcutaneous injection of one c. cm. of a fresh supply of normal serum from the same goat, collected with the same precautions as before. This was repeated every third day till the close of the experiment. The reason for reducing the dose was that the injection of the larger amounts of serum in the first series of animals produced much local disturbance. Two rabbits were given serum alone without bacilli, so as to act as controls. During the course of the investigation daily temperatures were taken and the animals were weighed weekly. A few of the animals died spontaneously before the six weeks allotted to the experiment had elapsed, but the remainder were killed in pairs on the same day A post-mortem was made immediately under strict aseptic conditions. In estimating the amount of disease resulting, I took into consideration the dissemination of the disease in the various organs, the amount of tissue destruction, the amount of repair, if any, the histological appearance of the lesions, and the morphology of the bacilli found. This necessitated the preparation of about two hundred microscopic preparations. The table herewith subjoined will give a general idea of the conditions resulting:—

Nos.	T. BEFORE INOC.	T. AFTER.	WT. BEFORE INOC.	WT. AFTER.	REMARKS.
G—Pigs.					
I.		104·2		274·5	Adv. tbc.
IV.	102·5	103·8	343·5grms.	329·4	Ditto.
II.	102·4	103·6	413	397	Adv. tbc.
III.	102·3	103·9	459	351	Ditto.
V.	102·8	103·3	359·5	310·2	Mod. tbc.
VI.	102·4	103·6	356·5	348·	Less adv. tbc.
Rabbits.					
I.	102·9	104	1779·5	1999·6	Slight tbc.
II.	103	103·6	1992	1979·4	Ditto.
III.	102·2	103·6	1604	1665·2	Tbc. minimal.
XII.	102·5	102·5	1643	1853·6	Slight tbc.
IV.	103·2	103·5	1301·5	1650·8	Mod. tbc.
XI.	101·9	102·7	1308·5	1458·2	Slight tbc.
V.	101·8	103·7	1717	1880	Slight local.
VII.		103·5		1836·4	Less marked.
VIII.	102·5	103	2130	2280·6	Mod. tbc.
X.	102	103·2	2199·5	1949·2	No tbc.
VI.	101·7	102·9	1781	1999·4	Control.
IX.	103·1	103·3	1286·5	1676·4	Control.

N. B.—In this table animals are arranged in pairs. G—P V and VI and R. III, V, VII and XII inoculated in leg, others intraperitoneally. Nos. in italics were given serum. Weight and temperature average.

Abstracts of autopsy protocols:—

GUINEA-PIG I.

Inoculated with one-half c.cm. of a standardized emulsion in saline of B. Tuberculosis intraperitoneally. Given normal goat serum, one c.cm. every third day, beginning June 9th. Died on twentieth day.

Site of inoculation in abdominal wall a large caseous sore, not, however, from tuberculosis. Abdominal wall contained numerous miliary and confluent tubercles. Abdominal cavity contained much blood-stained fluid in which the B. Tuberculosis was demonstrated. Right pleural cavity ditto, but no bacilli. Extensive caseous tuberculosis in peritoneal membrane, retroperitoneal, perigastric, mesenteric, and anterior mediastinal glands.

Spleen much enlarged and almost full of confluent caseous tubercles. Liver full of miliary tubercles. Other organs free. Smears from the spleen, liver, and anterior mediastinal glands gave B. Tuberculosis. Cultures from heart-blood and spleen, sterile.

Microscopic examination. *Heart*: cloudy. *Lung*: Slight acute bronchitis, collapse and hæmorrhagic infarction, slight leucocytic infiltration in septa. *Spleen*: Large coalescing tubercles with central necrosis, and chromatolysis. Cells chiefly lymphocytes. No attempt at fibrosis. *Liver*: Numerous large miliary foci, of which the largest showed slight caseation. The tubercles were in the portal sheaths, and were composed chiefly of lymphocytes together with fairly numerous epithelioid cells staining well. No fibrosis. The remaining liver cells cloudy and fatty. *Kidney*: Cloudy. *Lymph-gland* (1): Large caseous foci with chromatolysis, very few epithelioid cells and no fibrosis. *Lymph-gland* (2): Similar, but with slight proliferation of fibrous tissue at the periphery of the caseous foci. *Abdominal wall*: Section showed the muscle to be cloudy and in parts infiltrated with lymphocytes and numerous epithilioid cells. No caseation nor fibrosis.

Guinea-pig IV.

Inoculated intraperitoneally. On twenty-first day some induration of the abdominal wall was observed and in two days more a small pustule formed. In three days this opened spontaneously, and smears gave moderate numbers of tubercle bacilli. Killed July 30th.

At site of inoculation were numerous pearly and caseous masses in the abdominal wall and over the peritoneal membrane generally. Great omentum converted into a thickened caseous mass. Spleen and liver much enlarged and riddled with miliary tubercles. The diaphragm presented numerous pearly tubercles. A few retroperitoneal and numerous mesenteric glands enlarged and caseous. Lungs showed miliary tubercles; the anterior mediastinal glands enlarged and caseous. Kidneys, cloudy. Smears from the mesenteric glands and peritoneal fluid, negative.

Microscopic examination. *Heart*: Cloudy. *Lung*: Fairly numerous tubercles in the perivascular lymph spaces, consisting of lymphocytes and a few leucocytes. No caseation nor fibrosis. Parts of the lung showed congestion and alveolar catarrh. *Spleen*: Great hyperplasia and large caseous masses. Slight overgrowth of stroma. One giant-cell seen. *Liver*: Numerous and rather large early tubercles in the portal sheaths consisting mainly of lymphocytes. A few giant-cells seen, but no fibrosis. *Lymph-gland*: markedly caseous, but without fibrosis.

An interesting feature in this animal was that it aborted about one month before death and the uterus was enormously enlarged and caseous, while caseous matter had been discharged per vaginam.

Guinea-pig II.

Inoculated intraperitoneally. In twenty-one days a very small lump formed at the site of the inoculation. Died on the twenty-fourth day.

A small caseous dot found in abdominal wall at the site of inoculation. Hæmorrhagic peritonitis. Clear fluid found in both pleural sacs. The diaphragm showed no tubercles. Spleen very large, hæmorrhagic, and filled with caseous masses. Liver much enlarged and filled with what appeared to be caseous infarcts. Right lung contained a small caseous point surrounded by a congested zone. Anterior mediastinal glands enlarged and caseous. One or two retroperitoneal glands and a few inguinal ones enlarged and possibly tuberculous. Small pearly tubercles along vessels of mesentery of small intestine.

Great omentum, nodular and caseous. Other organs normal. Smears from the spleen, liver, and anterior mediastinal gland gave B. Tuberculosis; from peritoneal and left pleural fluid, negative. Cultures, sterile.

Microscopic examination. *Heart*: Cloudy. *Lung*: Some alveolar catarrh, numerous lymphocytes and leucocytes in the septa and alveolar walls, with slight cellular exudation into the alveolar spaces. *Spleen*: Enlarged, containing large confluent caseous masses with nuclear fragmentation. Exudation chiefly lymphocytes. No fibrosis. *Liver*: Numerous large tubercules in the portal sheaths, consisting mainly of lymphocytes, with in places caseation. Ischæmic necroses. Liver cells cloudy and fatty. *Lymph-glands* showed numerous small masses of caseation without peripheral fibrosis. *Abdominal wall*: Muscle fibres swollen and cloudy, with masses of lymphocytes and fairly numerous epithelioid cells between the muscle bundles. No fibrosis. Suprarenal and kidney, cloudy.

GUINEA-PIG III.

Inoculated intraperitoneally. *Given one c.cm. of goat serum every third day.* Died in twenty days. Broken tooth and infected jaw. Caseous focus in the abdominal wall at the site of inoculation, containing B. tuberculosis. Numerous small tubercles on lower portion of the peritoneum. Smears from these gave B. Tuberculosis. Great omentum contained large caseous nodules. Spleen and liver contained miliary tubercles. Films gave the specific bacillus. Diaphragm on under surface was covered with miliary tubercles. The anterior mediastinal glands enlarged and caseous, and a few retroperitoneal glands in the lumbar region enlarged, reddened, and showing beginning necrosis. Other organs free. Broth cultures from the heart-blood remained sterile.

Microscopic examination. *Heart* and *kidneys*: Cloudy. One minute patch of hæmorrhage with alveolar desquamation in the *lung* with but few leucocytes. *Spleen*: Follicles congested, pulp hyperplastic. Some large caseous areas without fibrosis. *Liver*: Cloudy and congested, with some fatty degeneration. Small miliary tubercles present, in only one of which was there beginning central caseation. A few epithelioid cells. No fibrosis. *Great omentum*: Full of caseous masses; one giant-cell seen. *Suprarenal*: Congested; a very small area of interstitial inflammation. *Abdominal wall*: Muscle fibres dissociated by inflammatory products, cloudy and disintegrated. Caseating tubercles with abundant lymphocytes fairly numerous epithelioid cells and polymorphonuclear leuccocytes. No fibrosis.

GUINEA-PIG V.

Inoculated in leg. *Given goat serum.* On twenty-first day nodule with infiltration of muscle at the site of inoculation. Killed on twenty-sixth day.

A caseous mass the size of a filbert found at site of inoculation. Two inguinal glands on left side enlarged and caseous and one on the right. Retroperitoneal glands similar. Two or three mesenteric glands caseous. Liver and spleen full of caseous masses. Minute tubercles in the great omentum, not caseous. Lungs showed miliary tubercles, and one or two of the anterior mediastinal glands enlarged, but not caseous. Kidneys, normal. Smear from the retroperitoneal glands gave B. Tuberculosis. Cultures from the heart-blod and inguinal glands gave staphylococci, others sterile.

Microscopical examination. *Heart* and *kidneys*: Cloudy. *Lung*: fairly numerous miliary tubercles, some just beginning to caseate. No fibrosis. *Spleen*: Very extensive caseation. *Liver*: Numerous and large tubercles without caseation in the portal sheaths, consisting of abundant lympocytes and fairly numerous epithelioid cells. *Various glands* showed caseous centres.

GUINEA-PIG VI.

Inoculated in leg. On twelfth day induration about site of inoculation, and one inguinal gland felt on the left side. On twenty-first day caseous focus point in leg. Two inguinal glands felt. On twenty-second day got cheesy pus from abscess which contained fragmented bacilli of Tuberculosis. Killed on the twenty-sixth day.

At site of inoculation, a caseous abscess. A few inguinal glands on the left side and several retroperitoneal glands enlarged and caseous. A few small pearly tubercles on peritoneum in lower portion, but relatively few on the diaphragm. Spleen enlarged, presenting a multitude of small pearly tubercles. Liver contained numerous small caseous foci. Kidneys free. A few mesenteric glands caseous. Lungs contain miliary tubercles. One anterior mediastinal gland enlarged. Omentum contained a few tubercles. Smears from the inguinal glands and spleen showed B. Tuberculosis. The amount of tuberculosis present was not nearly so great as in pig V.

Microscopical examination. *Heart* and *kidneys*: Cloudy. *Lungs*: Acute congestion with infarction. Fairly numerous tubercles, one or two extensively caseated, composed chiefly of lymphocytes. *Spleen*: Fairly numerous small tubercles with only slight caseation. *Liver*: Numerous large tubercles in the portal sheaths without caseation, consisting of numerous lymphocytes and fairly abundant epithelioid cells.

RABBIT I.

Inoculated intraperitoneally. *Given serum.* Killed on fiftieth day. One caseous retroperitoneal gland was found just below right kidney, but smears from this did not give tubercle bacilli. A small caseous tubercle in base of right lung. Cultures, sterile.

Microscopic examination. *Lung*: Infiltration with lymphocytes and large mononuclear cells in septa about the great vessels. Other organs cloudy or normal.

RABBIT II.

Inoculated intraperitoneally. Killed on fifty-first day. One or two small caseous masses on surface of the large bowel, films from which gave the B. Tuberculosis. A few caseous looking streaks along the lymphatics of the mesentery. Two small retroperitoneal glands enlarged. Cultures, sterile.

Microscopic examination. *Nodules* from *bowel*: Caseous tubercles composed chiefly of lymphocytes without giant-cells. *Liver*: Congested, numerous polymorphonuclear, mononuclear cells and phagocytes containing pigment and fragments of cells in the spaces between the liver cells. Other organs, cloudy.

RABBIT III.

Inoculated in leg. *Given serum.* On twelfth day one inguinal gland felt. Killed on fifty-fourth day. No signs of tuberculosis at site of inoculation. One or two glands found enlarged in the left leg, but no caseation elsewhere. Positive evidence of presence of tuberculosis not found. Cultures, sterile. Microscopical examination. Organs, cloudy.

RABBIT XII.

Inoculated in leg. On twenty-first day a small hard papule at site of inoculation. Killed on forty-fourth day. Inflammatory infiltration with caseation was found along the track of the inoculation, film from which gave moderate numbers of tubercle bacilli. Three or four retroperitoneal glands on the left side were enlarged and appeared to contain minute tubercles, films, however, did not reveal the bacillus. A few minute pearly tubercles were found in the mesentery along the course of the vessels. One or two mesenteric glands slightly enlarged.

Microscopical examination. Site of inoculation: Muscle fibres swollen, cloudy, dissociated, and disintegrated. Considerable caseation with abundant lymphocytes. Epithelioid cells were rather more numerous than in the case of the pigs.

RABBIT IV.

Inoculated intraperitoneally. Killed fifty-first day. Small caseous mass at site of inoculation. Numerous minute pearly tubercles noted on posterior part of peritoneum low down; also in lesser omentum and over stomach. Liver contained scanty pin-point tubercles. Lungs contained fairly numerous caseous tubercles the size of millet seeds. Smears from the lung and omentum gave B. Tuberculosis. Culture from blood, sterile.

Microscopical examination. *Lung*: Several miliary tubercles composed chiefly of epithelioid cells with rare lymphocytes. No caseation nor fibrosis. *Spleen*: hyperplasia of follicles. *Liver*: Contained miliary tubercles of small size, composed chiefly of epithelioid cells with a few lymphocytes at the periphery.

RABBIT XI.

Inoculated intraperitoneally. *Given serum.* Died on forty-first day. Much emaciated. The base of the left lung was rather reddened and contained a large miliary tubercle. A smear from this gave the specific bacillus. A few whitish miliary foci found elsewhere in the lung.

Microscopical examination. *Lung*: Showed several rather diffuse areas of inflammatory infiltration composed of lymphocytes and epithelioid cells. No caseation nor fibrosis. *Spleen*: Slight hyperplasia, contained numerous phagocytes with blood pigment. Other organs negative. This rabbit apparently died from the effect of the inoculations which had brought about some hæmolysis.

RABBIT VII.

Inoculated in leg. On twenty-first day a very small papule felt at site of inoculation. Killed on fifty-fifth day. Abscess with puriform matter and numerous tubercles at site of inoculation, containing numerous tubercle bacilli. One or two mesenteric glands enlarged. Culture from blood, sterile.

Microscopic examination. *Site of inoculation*: Caseous tuberculosis with rather numerous epithelioid cells, lymphocytes and polymorphonuclears. One or two giant-cells present. *Mesenteric gland* showed simple hyperplasia.

RABBIT V.

Inoculated in leg. *Given serum.* On twelfth day two glands enlarged in left inguinal region and one on the right. Killed on fifty-fifth day. A collection of tubercles found on the fascia of the left leg at site of inoculation, extending up the leg along the lymphatics. Rather dry. One retroperitoneal and two mesenteric glands enlarged.

Microscopical examination. All organs normal, or at most cloudy. Cultures from blood, sterile.

RABBIT VIII.

Inoculated intraperitoneally. On twenty-first day slight induration at site of inoculation. Killed on the fifty-first day. Infiltration the size of a pea at the site of inoculation which gave B. Tuberculosis. The psoas muscle on the right side inflamed, reddened, and on cutting into it it appeared caseous. Numerous pearly miliary tubercles scattered over peritoneum, mesentery, and omentum. Diaphragm showed a few miliary tubercles. A few retroperitoneal glands enlarged and caseous. A few rather large tubercles in the lower lobe of the right lung; one or two in right. Possibly a few minute tubercles in the liver. Spleen enlarged, but not visibly tuberculous. One or two mesenteric glands enlarged. A smear from the abdominal wall and also from the spleen gave the B. Tuberculosis. Culture from blood, sterile; from retroperitoneal gland, staphylococcus.

Microscopical examination. *Lung*: one or two small miliary foci composed of lymphocytes and epithelioid cells, without caseation. Small round-celled infiltration in the septa. *Spleen*: Congested, hyperplasia of follicles. *Liver*: A few minute collections of lymphoid cells with slight fatty degeneration. *Section of Wound*: Chronic granulation, with fragmentation and swelling of the muscle fibres.

RABBIT X.

Inoculated intraperitoneally. *Given serum.* On twenty-first day slight induration at the site of inoculation. Died on forty-seventh day. Large subcutaneous abscess in right flank caused by the inoculations. All organs found normal, except for congestion or cloudiness.

It was found in the course of this experiment that after the injection of the bacilli the average temperature of the animals was raised one degree. The average temperature of all the animals, both pigs and rabbits, was 102.52° before inoculation: after, it was 103.41° in those animals not receiving serum and 103.62 in those given it. In the case of the control animals that were given serum alone (Nos. VI and IX.), the temperature in one was only slightly elevated, in the other normal. We may thus conclude that the injection of the serum had no effect on the temperature curve. With regard to the weights the result was different. The animals receiving serum lost 22.27% of their body weight; those not receiving it lost only 10.15%. As a rule rabbits inoculated with tuberculosis preserve their nutrition surprisingly well until towards the last when they go down hill rather rapidly. The injection of the serum, although given in less than half the quantity employed in the first instance still caused considerable local disturbance, and this was aggravated by the animals scratching themselves so that the loss of weight is no doubt to be attributed to the interference with their feeding and the general irritation.

In comparing the results I found, as was expected, that the guinea-pigs are much more susceptible to tuberculosis than are rabbits, losing weight rapidly from the first and presenting marked lesions when killed. These facts led me to keep the rabbits under observation some three weeks longer, in the hope that thus the resulting disease would be more pronounced. This however did not prove to be the case.

After a careful consideration of the extent and nature of the lesions produced in the pigs it could not be said that the injection of the goat serum had the slightest effect in inhibiting the action of the bacilli. The results in the case of the rabbits were rather more promising. The most marked difference was found in rabbits III. and XII. Number three which had been given serum presented no positive appearance of tuberculosis, while its mate, number twelve, presented caseation at the site of inoculation and tubercles on the peritoneum. On the whole the lesions were slightly more marked in the case of the rabbits not receiving the serum. In corroboration of this finding may be cited the results of the first experiment where the only two animals that developed tuberculosis were those that had not been given serum. It is of course hazardous to draw too positive conclusions from such a small number of animals but it would appear so far as we have gone that normal goat serum does have a slight retarding effect on the progress of tuberculous infection. Whether this action is specific or not is another question. Recent work has shown that the serum of other animals, such as the dog, the ass, and the horse, as well as normal saline solution possess similar properties.

THE EFFECTS OF SERUM OF IMMUNIZED GOATS.

Having drawn this conclusion it was thought advisable to attempt to confer upon the serum more active antitoxic properties. The method adopted was based on that employed in the production of diphtheria antitoxin, namely the introduction of the toxins of the bacillus into the system of an animal until it was immune to the effects, and then using its serum as a curative agent. As has been pointed out most of the work on these lines has proved to be a failure or at most has had a very limited meed of success. This is possibly due, at least in part, to the fact that the toxins and extracts of the tubercle bacillus used for immunizing purposes have been obtained by heat or by various chemical processes so that they do not represent the full toxic properties of the bacillus. To obviate this objection Koch's new tuberculin (Bacillenemulsion) was employed. Perhaps a word or two of explanation as to the nature of this substance may not be out of place at this juncture.

Koch takes a definite weight of tubercle bacilli, filters them from all culture fluid, grinds them up with two hundred parts of 1/50 normal soda solution, and then centrifugalizes. He then pours off the supernatant fluid, adds weak acid to the residue until only slightly alkaline, and finally dilutes with a standard weak solution of carbolic acid and salt to the extent of one to three thousand. Glycerine is also added, and the final emulsion represents five milligrammes of pulverized bacilli in every cubic centimetre. (Deutsche med. Woch., Nov. 28, 1901). The injection of this into tuberculous persons brings about a rise of temperature of one and a half to two degrees centigrade. The dose of the first injection is 0.0025 milligramme rapidly increased two or five-fold until the reaction appears.

To obtain convenient amounts for injection, the bacillus emulsion was diluted according to Koch's directions with a standard diluting solution containing 0.8% sodium chloride and 0.5% carbolic acid.

Three strong healthy goats were subjected to the injection of the bacillus emulsion in gradually increasing amounts, the whole procedure extending over about seven months. The reason for spreading the injections over so long a period is that it had been found by Maragliano and others that the animals stand it better and the results are more satisfactory. It was determined beforehand by culture experiments that the emulsion was sterile. The injections were given subcutaneously in the neck under strictly aseptic conditions once a week until towards the end of the allotted period. Previously, however, the normal temperature for the goat was ascertained. The amount of the emulsion injected was at first .0025 milligrammes repeated once a week for three weeks and cautiously increased until at the end of three months the goats were receiving 0.015 milligrammes. Subsequently the amount injected was doubled each week, until at the end of seven months 15 milligrammes was reached. After the first three months also the temperature before inoculation was taken as well as afterwards, twice in the following twenty-four hours. The normal temperature of the goat varies between 101 and 103 degrees Fahrenheit. In only one case did the injection (of 10 mmg.), cause a rise of temperature from 102° to 103° and 3-5ths, but this was only 3-5ths of a degree above the maximum normal variation. The subsequent injection was lessened to 7.5 mmgs., and then again increased. During the last few weeks while such large amounts were being employed the injections were only given once in from ten to fourteen days. After the animals were considered immune to the emulsion a period of three weeks was allowed to elapse, until all excess of the toxin should have been eliminated from the system. One of the goats was then bled from the jugular with the same precautions as before adopted and the serum used for the purposes of the experiment. Tested by the Arloing-Courmont method as to its powers of agglutinating a homogeneous culture of the tubercle bacillus (kindly furnished me by Prof. Courmont), it gave a positive reaction in a dilution of one to fifty.

This power of agglutimation might, no doubt, have been greatly increased, as has been shown by Koch, but it was deemed sufficient for the immediate purposes of the experiment.

Experiment III.

In carrying out the third experiment I have laboured under considerable difficulties. Owing to the great disturbance caused by the injection of the serum in guinea-pigs it was thought better to use rabbits exclusively. Ten rabbits were taken, their temperature was noted daily for a week to establish a normal average, and their weight was recorded. They were then grouped in pairs according to their weight. Four were injected intravenously through the auricular vein; four intraperitoneally; and two in the left leg, with one half c. cm. of an emulsion of a mild tubercle bacillus in saline solution standardized as before. One member of each pair then received one c. cm. of the fortified goat serum. Unfortunately, after the experiment was well started, rabbit septicæmia broke out in the hutches and about half of the animals had to be replaced. At the end of a month several of the remaining animals were killed but it was found that the germ was not virulent enough to produce characteristic lesions. The animals were therefore reinoculated with the same quantity of an emulsion made from a mild germ received from Dr. DeSchweinitz, of the Bureau of Animal Industry, Washington. In addition two other rabbits were inoculated in the anterior chamber of the eye, affording a convenient means of watching the progress of the tuberculous infection. At the end of another month four rabbits were killed and again no lesions were discovered, a condition of things that was a little surprising when it was found that the disease progressed steadily although slowly in the case of those inoculated in the eyes. The results of more than two months' work was almost nil, although it served to indicate the effect produced by the antitoxic serum on the healthy organism. The average temperature before inoculation of the rabbits which did not receive serum was 102.9° and the average weight 1,865 grms. After the injection of the tubercle germs the average temperature was 102.7° and the weight 1,878 grms. The average temperature before inoculation with tuberculosis of the rabbits that did receive serum was 103.2° and the average weight was 1360 grms.; after inoculation with tuberculosis and after receiving antitoxic serum the average temperature was 103.2° and the weight 1673. Thus, as the culture inoculated was innocuous, the conclusion is that the antitoxic serum had no effect on the temperature while it apparently stimulated nutrition as the animals receiving it had markedly increased in weight, and in truth appeared in fine condition. This latter result, so different from that of the first two experiments, is explained from the fact that by a modification of the method the injections produced but little local disturbance, and the amount of irritation was reduced to a minimum. Finally as the experiment had to be concluded rather hastily, six guinea-pigs were taken, their normal temperature ascertained, and they were grouped in pairs as before according to weight. Two were inoculated in the left leg with a standardized emulsion of relatively mild bacilli (one c. cm.), and the remaining four intraperitoneally with the same amount. One member of each pair was given one c. cm. of antitoxic serum subcutaneously every second day. Numbers III. and VI., inoculated in the leg, died on the second day of the experiment, and presented no evidences of tuberculosis. Number IV. died on the ninth day and its mate was killed on the eleventh. Numbers I. and V. were killed on the fourteenth day.

Autopsy protocols: —

GUINEA-PIG I.

No signs of tuberculosis at the site of inoculation in the abdominal wall. No peritonitis. Two retroperitoneal glands on the left side were slightly enlarged but not caseous. Films from these did not show the bacillus tuberculosis. *Spleen*: Slightly enlarged and containing fair numbers of small greyish dots resembling early tubercles; films from the spleen, however, did not reveal the specific bacillus. A few small pearly nodules of greyish colour seen in the great omentum. A small yellow streak was seen in the *liver* not unlike a tubercle, but a smear from this did not show the tubercle bacillus.

Microscopical examination. *Great omentum*: The greyish dots referred to proved to be early tubercles. *Lung*: Slight bronchitis and peribronchitis. *Liver*: Cloudy. *Spleen*: Marked hyperplasia of the Malpighian bodies; no obvious tuberculosis. *This animal had been given serum.*

GUINEA-PIG V.

Mate of the last, receiving no serum. No signs of tuberculosis at site of inoculation in the abdominal wall. Two small retroperitoneal glands were in evidence, not caseous. One tubercle on right *testis*. A few mesenteric glands were slightly enlarged. *Spleen* much enlarged and full of whitish-grey dots the size of pin-heads resembling tubercles. Smears from the spleen gave tubercle bacilli.

Three isolated tubercles the size of hemp-seeds noticed in the liver. Films from these showed the specific bacillus. The *great omentum* was notably thickened, being converted into a transverse cord of firm gelatinous appearance. The pleural cavities contained a fair quantity of rather blood stained fluid. In this case the amount of tuberculosis was greatly in excess of that in the last animal.

GUINEA-PIG II.

Given serum. Slight redness and œdema at the site of the injection of the serum. No signs of tuberculosis at the site of inoculation in the abdominal wall. Two retroperitoneal glands near the pelvis swollen, but not caseous; films gave a few tubercle bacilli. Some minute pin-point nodules of pearly grey appearance were found in the *omentum.* Two larger nodules also found in the omentum, one of which was caseous. Other organs normal.

Microscopical examination. *Abdominal wall*: Considerable extravasation of leucocytes about the blood-vessels. Infiltration between the muscle fibres of leucocytes and considerable numbers of epithelioid cells. Muscle-fibres cloudy. No caseation. *Retroperitoneal gland*: Hyperplastic, but not caseous. *Liver*: Fatty, one or two minute areas composed of, in the main, clear mononuclear cells, resembling epithelioid cells. *Spleen*: Much congested with hyperplasia of the Malpighian follicles; no caseation. *Kidney*: Normal. The nodules in the *great omentum* proved to be early tubercles.

GUINEA-PIG IV.

Two glands enlarged but not caseous under the skin near the site of the inoculation, with congestion of the vessels in the neighbourhood. One retroperitoneal gland on the left side near the pelvis considerably enlarged and caseous. A film from this gave the B. Tuberculosis. A few mesenteric glands near the cœcum enlarged but not caseous. In the *great omentum* were numerous small gelatinous tubercles and caseous foci. Other organs free.

Microscopical examination. *Liver*: Congested and cloudy. *Kidney*: Cloudy. *Lung*: A few small well defined rounded areas about the vessels composed mainly of cells of epithelioid type, but with a few leucocytes; no caseation. *Spleen*: Considerable blood-pigment noticed. Hyperplasia of the Malpighian follicles. *Retroperitoneal gland:* Fairly extensive central caseation. *This animal had not been given serum.* Comparing this animal with its mate (No. II.), the amount of tuberculosis was notably greater, and this was even more marked when we take into account that the duration of the disease was two days less.

While this experiment was in progress the two rabbits that had previously been inoculated in the anterior chamber of the eye were kept under observation. One apparently had received some secondary infection at the operation as the eye was very rapidly destroyed and no safe conclusions could be drawn from it. The other proved more satisfactory however. For about two weeks the small caseous mass in the anterior chamber resulting from the injection slowly enlarged to about twice its original size. With this there was considerable swelling and injection of the iris with exudation and marked conjunctivitis. One c. cm. of the fortified serum was then given every third day. Following upon this the acute iritis and conjunctivitis subsided, and during the next three weeks that the animal was kept under observation while the disease undoubtedly progressed and subsidiary tubercles formed, there were no evidences of acute trouble, and the progression of the tuberclosis was somewhat slow and indolent.

With regard to temperatures, the average of all the pigs was 102.4° before inoculation. After inoculation the average of those receiving serum was 102.3°; of those not receiving it, 102°.

CONCLUSIONS.

From these various results it would look as if the injection of the antitoxic serum exercised a certain amount of restraining influence upon the extent and development of the disease although it is equally clear that it was not powerful enough to neutralize the infection or prevent its extension. Under ordinary circumstances I would hesitate to draw these conclusions from such a small series of animals were it not for the fact that my results are in perfect accord with work previously done on somewhat analogous lines. As a result of this study, taken in the light also of the results of other observers, I am led to the following conclusions:—

(1) That normal goat serum when injected into guinea-pigs and rabbits is practically innocuous, only rarely producing any hæmolysis.

(2) When injected with care it appears to stimulate nutrition rather than otherwise.

(3) Normal goat serum possesses a slight amount of antitoxic power against tuberculosis in rabbits.

(4) This natural antitoxic power may be considerably increased by injecting the goat with gradually increasing doses of Koch's New Tuberculin (Bacillenemulsion) until immune to its effects.

(5) Antitoxic serum produced in this way has some power in retarding tuberculous processes, although it is not strong enough to cure them.

(6) The injections of the antitoxic serum have no effect on temperature.

I believe, therefore, that it is possible to prepare an antitoxic serum for use in tuberculosis that will in a notable degree retard the disease in certain of the lower animals. Whether we shall be able to increase its power so as to bring about cure is another matter. In the case of the protective serum I have produced I did not expect even such good results as I obtained, for the reason that its agglutinative power was comparatively low (1—50) and it was possible to have increased this enormously. Possibly the serum of a goat treated by the tuberculin to saturation would give still better results. There would, however, be a limit to this procedure for the glycerine in Koch's emulsion would prove toxic when injected in any amount, producing hæmoglobinuria. Some method would therefore have to be devised whereby all the products of the tubercle bacillus could be injected without any foreign substances. In my opinion the various antituberculosis sera that have hitherto been produced have very questionable clinical value when applied to human beings. Of course after the announcement of any new curative agent there is always an outcry in its favour, but, in the case of tuberculosis, serum after serum has been placed upon the market only to be weighed in the balances and found wanting. The only serum that is still being used is that of Maragliano, and he is so convinced of its utility that an institute has recently been established in Italy for the treatment of tuberculosis by his method, to which he supplies serum gratis. The profession, however, appears to be strongly sceptical, for there is no rush to his laboratory from all parts of the world such as there was when Koch's ill-starred tuberculin was first announced. Maragliano's reported results, namely sixty per cent or thereabouts of improvements, are not much in advance of the figures furnished by any first class sanatorium, such as the Saranac Lake, Loomis, Muskoka, and Gœrbersdorf institutions, which are from forty to sixty per cent of cures in the early stages. It is moreover very difficult to estimate the value of any curative agent in the case of such a variable and insidious disease as tuberculosis. Many cases do well when placed in suitable surroundings with proper care and diet without drugs, and the disease is notoriously liable to unexpected remissions. The results of the serum treatment are not so good that we can yet abandon the attitude of expectant hope, nor should we pause in our attempts to obtain a still more potent serum. It may indeed well be that we have reached the possible limit of efficacy in the sera prepared by the methods hitherto adopted, and Koch is quite possibly right when he says that immunity to the action of the toxic products of the tubercle bacillus does not necessarily mean immunity to tuberculosis. We probably need some serum powerful enough to destroy the bacillus in the tissues or which, perhaps, will stimulate the body cells to elaborate a germicidal substance. The germicidal properties of the sera hitherto prepared do not appear to have been accurately observed and the subject will bear further study. Possibly advances in this direction will have to be made on the lines of Behring's recent work, who has succeeded in producing immunity in calves by injecting them with an attenuated human bacillus. If not, the problem will have to be attacked on quite different lines. We are confronted with a problem of no ordinary magnitude as every one will readily admit.

In conclusion I wish to express my thanks to the Hon. E. H. Bronson, of Ottawa, whose public spirit and generosity have made this research possible; to Dr. H. M. Kinghorn of Saranac Lake, and Dr. H. Wolferstan Thomas of Montreal, for cultures supplied; and to Professor J. G. Adami for many valuable hints during the progress of the investigation and his kindness in presenting this paper.

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